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ANTIPARASITIC DRUG

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ANTIPARASITIC DRUG

[Koukiseichuuzai]

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[There are no amendements to this patent.]

Claim /2*

An antiparasitic drug that contains as its active ingredient a peptide possessing an amino acid sequence disclosed in any of Sequence No. 1 through Sequence No. 31, a pharmaceutically permissible derivative of this peptide, a pharmaceutically permissible salt of this peptide, or a mixture of two or more of these.

Detailed explanation of the invention

[0001]

Technical field of the invention

The present invention pertains to an antiparasitic drug. More specifically, this inveniton pertains to a drug for the prevention and treatment of the various diseases that are produced in humans and animals, such as pets and livestock, by parasitic worms and protozoans in those animals. Furthermore, in the following disclosure, parasitic insects refers to parasitic organisms other than microorganisms, such as bacteria, fungi, and viruses, etc., and the term antibacterial activity refers to bacteriostatic or bacteriocidal action against microorganisms, such as bacteria, fungi, and viruses, etc.

[0002]

Prior art

Viewed worldwide, there is a high infection rate for parasitic diseases (from protozoans and worms), and large numbers of victims are still appearing in specific regions, or in regions not equipped with sanitation facilities. Livestock, such as cattle, sheep, pigs, goats, chickens, etc., and pets, such as dogs and cats, etc., also are susceptible to parasitic diseases. Parasitic diseases are diseases caused by worms, such as ascaris, nematodes, hookworms, pinworms, trichina, ciliates, cestodes, and trematodes, etc., and diseases caused by protozoans, such as malaria, toxoplasma, *Pheumocystis carinii*, and amoeba, etc., each parasites of which is characterized by its host and site of infestation.

[0003]

Even though parasites may not pose a grave threat to the life of their host, they cause major economic damage, especially in the livestock industry and the like by decreasing the nutritional efficiency and growth rate of animals, and by impacting the production of milk and wool, etc. Parasitic infestation of pets also is a major problem to the people who are intimately connected to those pets. Repellents, e.g., pyrimidine derivatives such as pyrantel pamoate,

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* [Numbers in right margin indicate pagination of the original text.]

benzimidazole derivatives such as mebendazole, isoquinoline derivatives such as praziquantel, piperazine derivatives such as piperazine phosphate and dimethylcarbamazine citrate, and the like, and natiprotozoan agents, e.g., nitroimidazole derivatives such as metronidazole, pyrimidine derivatives such as sulfamethoxazole trimethoprim, alkaloid substances such as quinine, quinoline substances such as 8-aminoquinoline, and antibiotics such as tetracycline, and the like, are currently used against these parasitic diseases (The Japanese Journal of Clinical Medicine, pp. 513-520, 1991, and "1991 Handbook of Veterinary Drugs and Implements," Japan Society of Veterinary Medicine pp. 217-218, 1991). However, these drugs have undesirable flaws in their side effects, such as stomachache, nausea, diarrhea, and vomiting, etc., and contraindication for pregnancy, etc.

[0004]

Meanwhile, lactoferrin is an iron-binding protein present in the bodily fluids, such as the milk, saliva, urine, and mucous secretions, etc., of mammals, including humans, and is known to exhibit antibacterial action against harmful microorganisms, such as *e. coli, candida*, and clostridia, etc. (Journal of Pediatrics No. 94, p. 1, 1979). It is also known to have antibacterial action against staphylococci and enterococci at concentrations of 0.5 to 30 mg/mL (Journal of Dairy Science, No. 67, p. 606, 1984).

[0005]

Focusing on the antibacterial properties of lactoferrin, the inventors in this matter discovered that substances in which mammalian lactoferrin, apolactoferrin, or metal-saturated lactoferrin (collectively referred to hereinafter as "lactoferrins") are hydrolyzed with an acid or enzyme have none of the undesirable side effects (e.g., antigenicity), etc., while possessing stronger heat resistance and antibacterial properties than those of unhydrogenated lactoferrins, and then submitted a patent application (Japanese Kokai Patent Application No. Hei 5[1993]-320068).

[0006]

In addition, the inventors in this matter isolated peptides with strong antibacterial activity from lactoferrin catabolites and synthesized peptides, or peptide derivatives thereof, with the same amino acid sequences as these peptides, and submitted patent applications for an antibiotic peptide made from 20 amino acid residues (Japanese Kokai Patent Application No. Hei 5[1993]-92994), an antibacterial peptide made from 11 amino acid residues (Japanese Kokai Patent Application No. Hei 5[1993]-78392), an antibacterial peptide made from 5 amino acid residues

(Japanese Kokai Patent Application No. Hei 5[1993]-148296), and an antibiotic peptide made from 3-6 amino acid residues (Japanese Kokai Patent Application No. Hei 5[1993]-148295).

[0007]

The inventors in this matter further discovered that peptides, or peptide derivatives thereof, with the same amino acid sequences as substances in which lactoferrins are hydrolyzed with acids or enzymes have brain-protecting action (Japanese Kokai Patent Application No. Hei 4[1992]-327738), action to promote the propagation of fibroblasts due to the epidermal growth factor in hydrolyzed lactoferrins (Japanese Kokai Patent Application No. Hei 6[1994]-48955), and action promoting the production of nerve growth factor (Japanese Kokai Patent Application No. Hei 5[1993]-23557), and have already submitted patent applications. They have also disclosed a method for isolating and purifying lactoferrins from milk using a property of lactoferrin to bond to heparin (Japanese Kokai Patent Application No. Sho[1998]63-255299).

[0008]

Furthermore, the inventors in this matter discovered that peptides with the same amino acid sequence as lactoferrins and substances in which lactoferrins are hydrolyzed with acids or enzymes possess prophylactic and curative efficacy against parasitic diseases in aquatic animals (Japanese Kokai Patent Application No. Hei 7[1995]-145069). However, the fact that these lactoferrins, their catabolites, and peptides with the same amino acid sequence as the catabolites /3
are effective against parasitic diseases caused by protozoans and worms, etc., that infest humans, livestock, such as cows, sheep, pigs, goats, and chickens, and pets, such as dogs and cats was not already known, and there is absolutely no literature stating such facts.

[0009]

Problems to be solved by the invention

As is clear from the aforementioned prior art, there is anticipation for antiparasitic drugs with low side effects, but there currently are still no superior substances. The present invention addresses the aforementioned circumstances, and its purpose is to provide an antiparasitic drug that posssesses prophylactic and curative effect against parasitic disaeses in humans, livestock, and pets, that is effective at low doses, and that has low side effects.

[0010]

Means to solve the problems

The present inveniton provides an antiparasitic drug that contains as its active ingredient a peptide possessing an amino acid sequence disclosed in any of Sequence No. 1 through

Sequence No. 31, a pharmaceutically permissible derivative of this peptide, a pharmaceutically permissible salt of this peptide (collectively referred to hereinafter as "peptides"), or a mixture of two or more of these.

[0011]

Conditions of application example of the invention

When manufacturing the peptides that are the active ingredients in the antiparasitic drug of the present inveniton from lactoferrins, lactoferrins used as the synthon are commercial lactoferrins, lactoferrins isolated by normal methods (e.g., ion-exchange chromatography) from mammalian (e.g., human, cow, sheep, goat, horse) colostrum, transitional milk, regular milk, and mature milk, etc., or products of these milks, e.g., skim milk or whey, etc., apolactoferrins that have been iron-depleted with hydrochloric acid or citric acid, etc., and metal-saturated or partially saturated lactoferrins, in which apolactoferrin has been chelated with a metal, such as iron, copper, zinc, or manganese, etc., and either commercial products or preparations manufactured by known methods may be used.

[0012]

The peptides used in the present invention are peptides obtained by isolation means from catabolites of lactoferrins, peptides with the same amino acid sequence or a homologous amino acid sequence of these peptides, deficatives of these peptides, pharmaceutically permissible salts of these peptides, or any mixture thereof, and can be chemically synthesized by commonly known methods. These peptides can be obtained by the methods disclosed in various patents, e.g., the aforementioned Japanese Kokai Patent Application No. Hei 5[1993]-92994, Japanese Kokai Patent Application No. Hei 5[1993]-78392, Japanese Kokai Patent Application No. Hei 5[1993]-148297, Japanese Kokai Patent Application No. Hei 5[1993]-1498296, and Japanese Kokai Patent Application No. Hei 5[1993]-148295.

[0013]

Peptides obtained by the aforementioned methods, peptides with the following amino acid sequences, and derivatives or salts thereof, can be given as examples of desirable embodiments. Examples include peptides with the amino acid sequences in sequence Nos. 1, 2, and 27 and salts or derivatives thereof (Japanese Kokai Patent Application No. Hei 5[1993]-78392), peptides with the amino acid sequences in sequence Nos. 3, 4, 5, and 6 and salts or derivatives thereof (Japanese Kokai Patent Application No. Hei 5[1993]-148297), peptides with the amino acid sequences in sequence Nos. 7, 8, 9, and 31 and salts or derivatives thereof (Japanese Kokai Patent Application No. Hei 5[1993]-148296), peptides with the amino acid

sequences in sequence Nos. 10-21 and salts or derivatives thereof (Japanese Kokai Patent Application No. Hei 5[1993]-148295), and peptides with the amino acid sequenses in sequence Nos. 22-26, 28, 29, and 30 and salts or derivatives thereof (Japanese Kokai Patent Application No. Hei 5[1993]-92994).

[0014]

Examples of pharmaceutically permissible salts of the aforementioned peptides include oxygenated salts, such as hydrochlorides, phosphates, sulfates, citrates, lactates, and tartrates, etc., and examples of the derivatives include derivatives in which a carboxylic group has been amidated or an amino group has been acylated. The resulting peptides have low toxicity, as shown in Test Example 4, and can be employed as drugs by commonly known methods as pills, capsules, lozenges, syrups, powders, dispersions, and injections, etc., and can be used as ointments, liquid applications, lotions, aerosols (sprays), and suppositories, etc., They further may also be administered mixed into food, animal feed, or drinking water, etc.

[0015]

The quantity of peptides that are the active ingredient in the antiparasitic drug of the present invention can be selected appropriately according to the disease, etc., but it is at least 0.1 mg, preferably 1 mg or more, per 1 kg of body weight for oral preparations, at least 0.02 mg, preferably 0.2 mg or more, per 1 kg body weight for injectable preparations, and 0.05 to 100 mg per 1 g for topical preparations.

[0016]

Test examples will be shown below to explain in further detail the effects of the peptides that are the active ingreadient in the antiparasitic drug of the present invention.

Test Example 1

This test was conducted to determine the parasiticidal effect of the peptides.

1) Test Methodology

A *Toxoplasma gondii* suspension (isloated according to Cornelisen's et al., method in Parasitology, Vol. 83, pp. 103-108, 1981) was added to a D-MEM 1% BSA (least medium required for Dulbecco modification containing 1% BSA) containing various concentrations, 0 μg/mL (control), 100 μg/mL, and 1000 μg/mL, of the peptide with Sequence No. 26, manufactured according to the method in Reference Example 1, and cultured for 30 minutes, 1 hour, 2 hours, or 4 hours at 37°C. After cultivation was completed, the parasites were collected

from the various media by centrifugal separation for 10 minutes at 1200×g, washed 3 times with phosphoric acid buffer solution (PBS), and resuspended in D-MEM 1% BSA to a parasite concentration of 1×10^6/mL. Equivalent amounts of PBS containing 0.5% trypan blue were added to 5 μL of each of these suspensions and the survival rates of *Toxoplasma gondii* were calculated microscopically.

2) Test Results

The results of this test are as shown in Figure 1. Figure 1 shows the survival rate of *Toxoplasma gondii*, wherein the vertical axis shows the parasite survival rate (percentage of unstained parasites), the horizontal axis shows the culture time, and ○, ●, and ▲ indicate the control, the medium containing 100 μg/mL of the peptide of Sequence No. 26, and the medium containing 1000 μg/mL of the peptide of Sequence No. 26, respectively.

[0017]

As is clear from Figure 1, while there was no change in the parasite survival rate up to 4 hours of cultivation, the parasite survival rates after 1, 2, and 4 hours of cultivation in the medium containing 100 μg/mL of the peptide of Sequence No. 26 were 64%, 51%, and 5%, respectively, and the parasite survival rates after more than 30 minutes of cultivation in the medium containing 1000 μg/mL of the peptide of Sequence No. 26 was less than 5%. From these results, the peptide of Sequence No. 26 was found to have parasiticidal effect. Furthermore, trials were performed with various other the peptides, but similar results were obtained.

Test Example 2

This test was conducted to determine the effect of the peptides at preventing parasitic invasion of cells, using mouse germ cells.

1) Test Methodology

After culturing mouse germ cells (prepared by Omata's et al. method (Parasitology Research, No. 75, pp. 189-193, 1990)) in D-MEM 10% FBS (least medium required for Dulbecco modification containing 10% FBS), they were incubated in PBS containing 0.025% trypsin, cells were collected by centrifuge for 10 minutes at 800×g, and then resuspended in D-MEM 10% FBS to a concentration of 5×10^4 cells/mL, which was then transplanted, 200 μL each, to culture plates (15 mm diameter, Matsunami Glass Co.) and cultured overnight at 37°C. 0.1 mL of culture solutions of *Toxoplasma gondii* that had been treated by the same method as in Test Example 1 for 0 hours, 15 minutes, 30 minutes, 1 hour, 2 hours, and 4 hours with the peptide of Sequence No. 26 were added to the culture solution of mouse germ cells, and then

incubated at 37°C. 18 hours later, the plates were washed with PBS, gelled with methanol, and then Giemsa-stained. The ratio of *Toxoplasma gondii* parasite cells per 500 mouse germ cells was then calculated to find the infection rate.

2) Test Results

The restuls of this test are as shown in Figure 2. Figure 2 shows the rate of infection of mouse germ cells by *Toxoplasma gondii*, wherein the vertical axis shows the respective rates of infection of mouse germ cells, the horizontal axis shows the time that the parasites were treated with the peptide of Sequence No. 26, and ○, ●, and ▲ indicate the control, the medium containing 100 μg/mL of the peptide of Sequence No. 26, and the medium containing 1000 μg/mL of the peptide of Sequence No. 26, respectively.

[0018]

As is clear from Figure 2, while the rate of parasitic infection in the control group was over 78% at up to 4 hours of treatment time, the parasitic infection rates after 15 minutes, 30 minutes, 1, 2, and 4 hours of treatment with 100 μg/mL of the peptide of Sequence No. 26 were 80%, 58%, 35%, 20%, and 8%, respectively, and the parasitic infection rates after 30 minutes and 1 hour or more of treatment with 1000 μg/mL of the peptide of Sequence No. 26 were 16% and less than 10%, respectively. From these results, the peptide of Sequence No. 26 was found to be effective at preventing parasitic invasion of cells. Furthermore, trials were performed with various other the peptides, but similar results were obtained.

Test Example 3

This test was conducted, similar to Test Example 2, to determine the effect of peptides at preventing parasitic infection, using mice.

1) Test Methodology

After treating suspensions of *Toxoplasma gondii* by a method similar to the test methodology in Test Example 1, by leaving them exposed for 4 hours to 0 μg/mL (control), 100 μg/mL, and 1000 μg/mL solutions of the peptide of Sequence No. 26, 100 *Toxoplasma gondii* were injected into the stomach cavities of mice in groups of 5 each. The survival of the mice was then monitored for the subsequent 30 days.

2) Test Results

The results of this test are as shown in Table 1. Table 1 shows the changes in the survival population of mice after injection of the parasites.

[0019]

As is clear from Table 1, while all 5 of the mice in the group injected with parasites that had not been treated with the peptide (control group) had died by 9 days after injection, all of the mice in each of the groups injected with parasites that had been treated with 100 μg/mL and 1000 μg/mL of the peptide of Sequence No. 26 had survived up to 9 days after injection, and 4 out of 5 of the mice in the group treated with 1000 μg/mL survived up to 30 days after injection. These results confirmed that the peptide of Sequence No. 26 had parasiticidal effect using actual live bodies. Furthermore, trials were performed with various other the peptides, and similar results were obtained.

[0020]

Table 1

Test Group	Survival Rate %	
	9 days after injection	30 days after injection
Control Group	0	0
100 μg/mL Treated Group	100	20
1000 μg/mL Treated Group	100	80

[0021]

Test Example 4

This test was conducted to determine the acute toxicity of the peptides.

1) Test Methodology

6-week-old CD (SD) rats (purchased from Nihon SLC) of both genders were divided into 4 groups (5 rats per group) to randomize the males and females.

[0022]

The peptide of Sequence No. 26, manufactured by the same method as in Reference Example 1, was disolved into injectable water (Otsuka Pharmaceutical Co.) to ratios of 1000 or 2000 mg per 1 kg body weight, of which 4 mL per 100 g body weight was then administered once by forced oral administration using a metal ball-point needle, to test acute toxicity.

2) Test Results

As a result of this test, there were no deaths in either of the groups administered 1000 mg/kg body weight or 2000 mg/kg body weight of peptide. Consequently, with an LD_{50} value of over 2000 mg/kg body weight, this peptide was deemed to have extremely low toxicity. Furthermore, trials were performed with various of the peptides, and similar results were obtained.

Reference Examplel 1

50 mg of commercial bovine lactoferrin (Sigma Co.) were dissolved in 0.9 mL purified water, after which, 1 mg commercial porcine pepsin (Sigma Co.), pH-adjusted to 2.5 with 0.1 normal hydrochloric acid, was added, and hydrolyzed for 6 hours at 37°C. Next, the pH was adjusted to 7.0 with 0.1 normal sodium hydrochloride, the solution was heated for 10 minutes at 80°C to deactivate the enzyme, cooled to room temperature and centrifuged for 30 minutes at 15,000 rpm to yield a clear supernatant. 100 μL of this supernatant was subjected to high-speed liquid chromatography using a TSK gel ODS-120T (Tosoh Co.), and after specimen injection at a 0.8 mL/min flow rate, was eluted for 10 minutes with 20% acetonitrile containing 0.05% TFA (trifluoroacetic acid), and then eluted for 30 minutes with a 20-60% acetonitrile gradient containing 0.05% TFA, the eluent fraction was collected for 24-25 minutes and vacuum-dried. This dried substance was then dissolved in purified water to a 2% (w/v) concentration, subjected again to high-speed liquid chromatography using a TSK gel ODS-120T (Tosoh Co.), and after specimen injection at a 0.8 mL/min flow rate, was eluted for 10 minutes with 24% acetonitrile containing 0.05% TFA, and then eluted for 30 minutes with a 24-32% acetonitrile gradient containing 0.05% TFA, the eluent fraction was collected for 33.5-35.5 minutes. The aforementioned operation was repeated for 25 times, followed by vacuum drying to yield approximately 1.5 mg of peptide.

[0023]

The aforementioned peptide was hydrolyzed with 6N hydrochloric acid, and then analyzed by the usual method for its amino acid composition, using an amino acid analyzer. When Edman sequence analysis was performed 25 times on identical samples using a gas phase sequencer (Applied Biosystems Co.), a sequence of 25 amino acid residues was determined. Disulfide bond analysis (Analytical Biochemistry, No. 67, p. 493, 1975) using DTNB [5,5-dithio-bis(2-nitrobenzoic acid)] also confirmed the presence of disulfide bonds.

[0024]

These results confirmed that this peptide possesses the amino acid sequence noted in Sequence No. 26, comprising 25 amino acid residues, wherein the No. 3 and No. 20 cysteine residues form a disulfide bond, and two amino acid residues toward the N-terminus side from the No. 3 cysteine residue, and the 5 amino acids toward the C-terminus side from the No. 20 cysteine residue, are bonded.

Reference Example 2

A peptide was synthesized as follows, based on the solid-phase peptide synthesis methods of Shepard et al. (Journal of Chemical Society, Perkin 1, p. 538, 1981), using an automatic peptide synthesizer (Pharmacia LKB Biotechnology Co., LKB Biolynx 4170).

[0025]

A desired amino acid anhydride was produced by adding N,N-dicyclohexylcarbodiimide to an amino acid whose amine functional group was protected by a 9-fluoronylmethoxycarbonyl group (hereinafter referred to as Fmoc-amino acid or Fmoc-intrinsic amino acid (e.g., Fmoc-asparagine)), which Fmoc-amino acid anhydride was used for synthesis. In order to manufacture a peptide chain, an Fmoc-asparagic anhydride equivalent to a C-terminus asparagine residue was set by means of its carboxyl group in Ultrosin A resin (Pharmacia LKB Biotechnology Co.), using dimethylamino pyridine as a catalyst. Next, this resin was washed with dimethyl formamide containing piperazine to remove the protective group from the amine functional group from the C-terminus amino acid. The Fmoc-arginic anhydride corresponding to the 2nd position from the C-terminus of the amino acid sequence was then coupled to unprotected amine functional group of arginine fixed in resin by means of the aforementioned C-terminus amino acid residue. Glutamine, tryptophan, glutamine, and phenylalanine were then sequentially fixed in the following manner. Once coupling of all the amino acids was completed and a peptide chain of the desired amino acid sequence had been formed, the protective groups except for acetoamidomethyl were removed and the peptide was released with a solvent made from 94% TFA, 5% phenol, and 1% ethane diol, the peptide was purified by high-speed liquid chromatography, and the resulting solution was concentrated and dried to yield a peptide powder.

[0026] /6

The amino acid composition of the aforementioned peptide was analyzed by the usual method using an amino acid analyzer to confirm that it possessed the amino acid sequence noted in Sequence No. 10. The present invention will be concretely explained in more detail below by

showing example embodiments, but the present invention is not limited to the following examples.

[0027]

Application Example 1

Pills were manufactured with the following formulation per pill.

peptide of Sequence No. 10	10.0 (mg)
lactose monohydrate	30.0
cornstarch	19.8
crystal cellulose	28.0
magnesium silicate pentahydrate	2.0
magnesium stearate	0.2

A mixture of the peptide of Sequence No. 10, manufactured by the same method as in Reference Example 2, lactose monohydrate, cornstarch, and crystalline cellulose were uniformly kneaded together while appropriately adding sterilized water, and then dried for 3 hours at 50°C. Magnesium silicate pentahydrate and magnesium stearate were then admixed into the resulting dried product and made into pills by the usual method using a pill press. Furthermore, the raw materials other than the peptide are all used in commercial products.

Application Example 2

10 mg of the peptide powder of Sequence No. 26, manufactured by the same method as in Reference Example 1, and 9 mg of sodium chloride (Wako Pure Chemical Industries, Ltd.) were dissolved into 1 mL of injectable water (Otsuka Pharmaceutical Co.); this was then pH-adjusted to approximately 7 using sodium hydroxide (Wako Pure Chemical Industries, Ltd.) and hydrochloric acid (Wako Pure Chemical Industries, Ltd.), filter-sterilized, and placed in 1 mL ampoules by the usual method to manufacture an injectable antiparasitic preparation.

Application Example 3

1 mg of the peptide powder of Sequence No. 10, manufactured by the same method as in Reference Example 2, and 49.5 mg D-mannite (Wako Pure Chemical Industries, Ltd.) were dissolved in 10 mL injectable water (Otsuka Pharmaceutical Co.); this was then pH-adjusted to approximately 7 with an aqueous solution of powdered phosphoric acid buffer (Wako Pure Chemical Industries, Ltd.), filter-sterilized, placed in 1 mL vials by the usual method and freeze-dried to manufacture an injectable antiparasitic preparation.

Application Example 4

An ointment of the following formulation per 100 mg was manufactured by the usual method. Furthermore, the raw materials other than the peptide are all used in commercial products.

[0028]

peptide of Sequence No. 26, manufactured by the same method as in Reference Example 1	0.1 (gm)
squaline	10.0
white petroleum jelly	8.0
cetostearyl alcohol	8.0
glycerin monostearate	2.0
polyoxyethylene monostearate	1.0
paraoxymethylbenzoate	0.2
paraoxypropylbenzoate	0.1
1,3-butylene glycol	2.5
sterile purified water	68.1

[0029]

Effect of the invention

As described in detail above, the present invention is associated with an antiparasitic drug that contains peptides as its active ingredient, and the effects realized by the present invention are as follows.

(1) Low side effects

(2) Heat resistant, soluble in water, and stable in aqueous solution, therefore stable as a drug preparation

(3) Since the peptide possesses antibacterial action, preservatives do not need to be used when produced as a drug preparation.

(4) Shows toxicity only toward parasites, without exhibiting cytotoxicity toward normal cells.

[0030]

Sequence list

Sequence No. 1

Sequence length: 11
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0031]
Sequence:

```
Lys R01 R01 R01 R01 Gln R01 R01 Met Lys Lys
1               5                  10
```

Sequence No. 2
Sequence length: 11
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys. /7

[0032]
Sequence:

```
Lys R01 R01 R01 R01 Gln R01 R01 Met Arg Lys
1               5                  10
```

Sequence No. 3
Sequence length: 6
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0033]
Sequence:

Arg R01 R01 R01 R01 Arg
1 5

Sequence No. 4
Sequence length: 6
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0034]
Sequence:
Lys R01 R01 R01 R01 Arg
1 5

Sequence No. 5
Sequence length: 6
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0035]
Sequence:
Lys R01 R01 R01 R01 Lys
1 5

Sequence No. 6
Sequence length: 6
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0036]

Sequence:

Arg R01 R01 R01 R01 Lys

1 5

Sequence No. 7

Sequence length: 5

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0037]

Sequence:

Arg R01 R01 R01 Arg

1 5

Sequence No. 8

Sequence length: 5

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0038]

Sequence:

Lys R01 R01 R01 Arg

1 5

Sequence No. 9

Sequence length: 5

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0039]

Sequence:

Arg R01 R01 R01 Lys

1 5

Sequence No. 10

Sequence length: 6

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0040]

Sequence:

Phe Gln Trp Gln Arg Asn

1 5

Sequence No. 11

Sequence length: 5

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0041]

Sequence:

Phe Gln Trp Gln Arg

1 5

/8

Sequence No. 12

Sequence length: 4

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0042]

Sequence:

Gln Trp Gln Arg

1

Sequence No. 13

Sequence length: 3

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0043]

Sequence:

Trp Gln Arg

1

Sequence No. 14

Sequence length: 5

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0044]

Sequence:

Arg Arg Trp Gln Trp

1 5

Sequence No. 15

Sequence length: 4

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0045]

Sequence:

Arg Arg Trp Gln
1

Sequence No. 16
Sequence length: 4
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0046]
Sequence:
Trp Gln Trp Arg
1

Sequence No. 17
Sequence length: 3
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0047]
Sequence:
Gln Trp Arg
1

Sequence No. 18
Sequence length: 6
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0048]
Sequence:
Leu Arg Trp Gln Asn Asp
1 5

Sequence No. 19
Sequence length: 5
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0049]
Sequence:
Leu Arg Trp Gln Asn
1 5
Sequence No. 20
Sequence length: 4
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0050]
Sequence:
Leu Arg Trp Gln
1
Sequence No. 21
Sequence length: 3
Sequence type: amino acid
Topology: straight chain
Sequence variety: peptide
Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0051]
Sequence:
Arg Trp Gln
1

Sequence No. 22
Sequence length: 20

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, the No. 2 Cys and the No. 19 Cys form a disulfide bond.

[0052]

Sequence:

```
Lys Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala Pro
1               5                  10                  15
Ser Ile Thr Cys Val
            20
```

Sequence No. 23

Sequence length: 20

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, Cys* indicates cysteine that has been chemically modified with a thiol group to prevent formation of a disulfide bond.

[0053]

Sequence:

```
Lys Cys* Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala Pro
1               5                  10                  15
Ser Ile Thr Cys* Val
            20
```

Sequence No. 24

Sequence length: 20

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, the No. 2 Cys and the No. 19 Cys form a disulfide bond.

[0054]

Sequence:

```
Lys Cys Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg Gly Pro
1               5                  10                  15
Pro Val Ser Cys Ile
            20
```

Sequence No. 25

Sequence length: 20

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, Cys* indicates cysteine that has been chemically modified with a thiol group to prevent formation of a disulfide bond.

[0055]

Sequence:

```
Lys Cys* Phe Gln Trp Gln Arg Asn Met Arg Lys Val Arg Gly Pro
1               5                  10                  15
Pro Val Ser Cys* Ile
            20
```

Sequence No. 26

Sequence length: 25

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, the No. 3 Cys and the No. 20 Cys form a disulfide bond.

[0056]

Sequence:

```
Phe Lys Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala
1               5                  10                  15
Pro Ser Ile Thr Cys Val Arg Arg Ala Phe
            20                  25
```

Sequence No. 27

Sequence length: 11

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment.

[0057]

Sequence:

```
Lys Thr Arg Arg Trp Gln Trp Arg Met Lys Lys
1               5                  10
```

Sequence No. 28

Sequence length: 38

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, the No. 16 Cys and the No. 33 Cys form a disulfide bond.

[0058]

Sequence:

```
Lys Asn Val Arg Trp Cys Thr Ile Ser Gln Pro Glu Trp Phe Lys
1               5                  10                  15
Cys Arg Arg Trp Gln Trp Arg Met Lys Lys Leu Gly Ala Pro Ser
                20                  25                  30
Ile Thr Cys Val Arg Arg Ala Phe
                35
```

Sequence No. 29

Sequence length: 32

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, the No. 10 Cys and the No. 27 Cys form a disulfide bond.

[0059]

Sequence:

```
Thr Ile Ser Gln Pro Glu Trp Phe Lys Cys Arg Arg Trp Gln Trp
1               5                  10                  15
Arg Met Lys Lys Leu Gly Ala Pro Ser Ile Thr Cys Val Arg Arg
            20                  25                  30
Ala Phe
```

Sequence No. 30

Sequence length: 47

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, the 36 protein-long peptide with a No. 9, No. 26, and No. 35 Cys, the No. 9 Cys and No. 26 Cys form a disulfide bond, and the No. 35 Cys in the aforementioned 36-protein-long sequence forms a disulfide bond with the No. 10 Cys in the 11-protein-long sequence with a No. 10 Cys.

[0060]

Sequence:

```
Val Ser Gln Pro Glu Ala Thr Lys Cys Phe Gln Trp Gln Arg Asn
1               5                  10                  15
Met Arg Lys Val Arg Gly Pro Pro Val Ser Cys Ile Lys Arg Asp
            20                  25                  30
Ser Pro Ile Gln Cys Ile
            35
Gly Arg Arg Arg Arg Ser Val Gln Trp Cys Ala
1               5                  10
```

Sequence No. 31

Sequence length: 5

Sequence type: amino acid

Topology: straight chain

Sequence variety: peptide

Sequence characteristics: This peptide and peptides that contain this peptide as a fragment. In the following sequence, R01 represents any amino acid residue except Cys.

[0061]

Sequence:

Lys R01 R01 R01 Lys

1 5

Brief description of the figures

Figure 1 shows the relationship between the parasite survival rate and the culture time.

Figure 2 shows the relationship between the rate of mouse germ cell infection by *Toxoplasma* and treatment time.

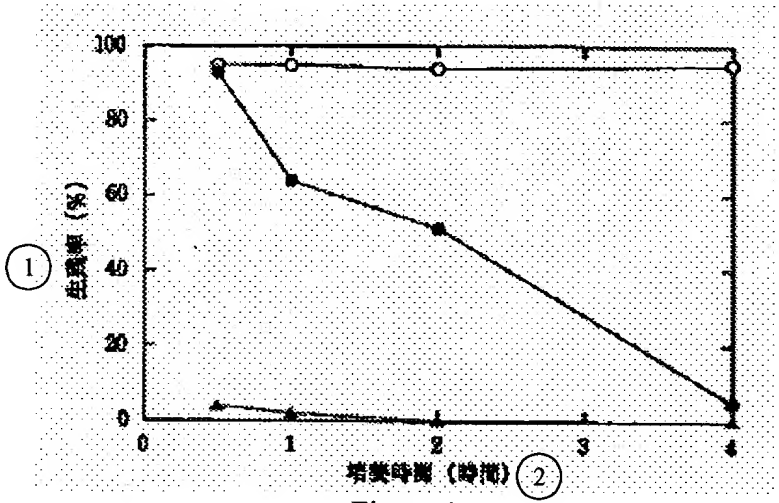

Figure 1

Key: 1 Survival Rate (%)
2 Culture Time (hours)

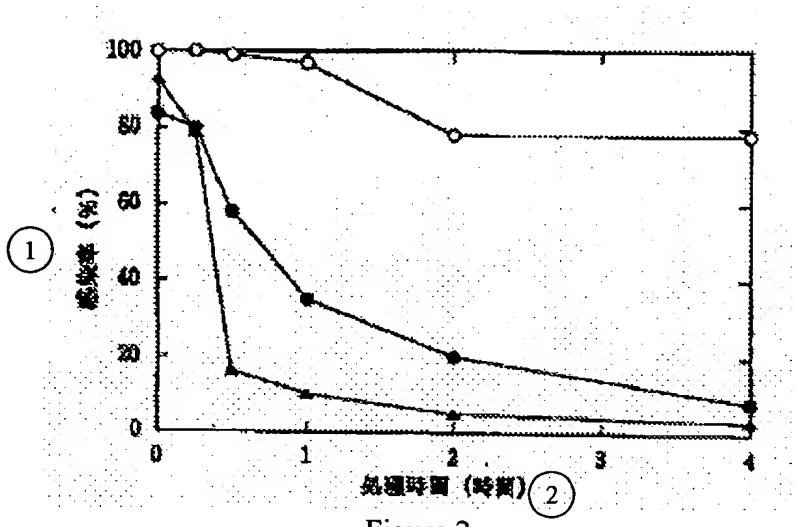

Figure 2

Key: 1 Infection Rate (%)
2 Treatment Time (hours)